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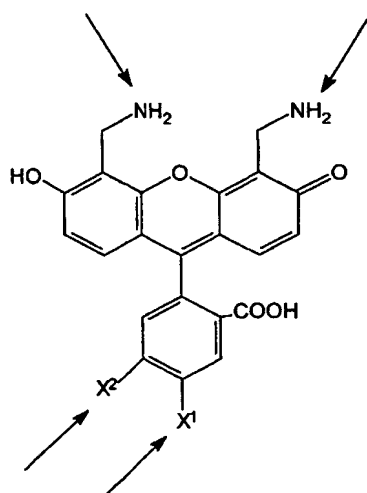
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(54) Title: **FLUORESCENT LABELING REAGENTS WITH MULTIPLE DONORS AND ACCEPTORS**



$x^1 = \text{COOH}, x^2 = \text{H}, \text{ or}$

$x^1 = \text{H}, x^2 = \text{COOH}$

The structure of 4',5'-bis-aminomethyl-fluorescein molecular building block and the preferred positions (arrowed) for possible attachment of other fluorophore(s), target bonding groups, solubilizing and charge carrying constituents and/or carrier material.

(57) **Abstract:** Disclosed is a novel class of fluorescent resonance energy transfer (FRET) labelling reagents, based on and synthesised from easily prepared dye building blocks. The labelling reagents are in the form of "cassettes" which enable their attachment to a wide variety of biological and other materials. A labelling reagent comprises at least two fluorescent dye moieties covalently linked via a linker group and optionally having a target bonding group for attaching the reagent to a target. The energy transfer labelling reagents may be bound to target materials through covalent or non-covalent attachment. The dyes are selected so that the emission spectrum of a first (or donor) dye overlaps the absorption spectrum of a second dye, thereby allowing energy transfer to occur between the dyes. The dye building blocks are 4', 5'-bis-aminomethyl-fluorescein and/or its 5(6)-carboxylic acid and having the structure (I). In addition to the embodiment of the invention which includes a single donor and a single acceptor fluorochrome, the fluorescent energy transfer labelling reagents according to the invention may further comprise one or more third fluorochromes each having third absorption and emission spectra covalently attached to said first or second fluorochromes.